REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	March 30, 2000	Final, 6/1/99 - 2/29/00

4. TITLE AND SUBTITLE

Assembling Nano-Materials by Bio-Scaffolding: Crystal Engineering in Nano-Electronics

5. FUNDING NUMBERS

DAAD19-99-1-0193

6. AUTHOR(S)

Geoffrey F. Strouse

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Department of Chemistry & Biochemistry
University of California
Santa Barbara, CA 93106

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Research Office
P.O.Box12211
Research Triangle Park, NC 27709-2211

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

40143.1-LS-II

11. SUPPLEMENTARY NOTES

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release, distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Developing non-lithographic techniques for assembly of nano-scale materials (metals, semiconductors) at the interface between nano-materials and biology offers a glimpse into the design of potential materials for optical or electronic devices. Materials assembly using biological polymers (DNA, proteins) as scaffolds to direct the assembly of nano-scale components (semiconductors, metals) offers an exciting possibility of blending the inherent self-assembling properties of biological materials and the unique electronic and optical properties of nanomaterials. We have investigated the assembly of nano-scale materials (Au, Ag, CdSe) into 3-d super-lattices of nano-particles connected by biological polymers (DNA, polypeptides), in which the biological spacers act as molecular level scaffolds for nano-assembly.

14. SUBJECT TERMS

nano-scale materials, biological polymers, electronic and optical properties of nanomaterials

15. NUMBER OF PAGES

8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

MASTER COPY: PLEASE KEEP THIS "MEMORANDUM OF TRANSMITTAL" BLANK FOR REPRODUCTION PURPOSES. WHEN REPORTS ARE GENERATED UNDER THE ARO SPONSORSHIP, FORWARD A COMPLETED COPY OF THIS FORM WITH EACH REPORT SHIPMENT TO THE ARO. THIS WILL ASSURE PROPER IDENTIFICATION. NOT TO BE USED FOR INTERIM PROGRESS REPORTS; SEE PAGE 2 FOR INTERIM PROGRESS REPORT INSTRUCTIONS.

MEMORANDUM OF TRANSMITTAL

U.S. Army Research Office
ATTN: AMSRL-RO-RI (Hall)
P.O. Box 12211
Research Triangle Park, NC 27709-2211

☐ Reprint (Orig + 2 copies)
☐ Technical Report (Orig + 2 copies)
☐ Manuscript (1 copy)
☒ Final Progress Report (Orig + 2 copies)
☐ Related Materials, Abstracts, Theses (1 copy)

CONTRACT/GRANT NUMBER: DAAD19-99-1-0193

REPORT TITLE: Assembling Nano-Materials by Bio-Scaffolding: Crystal Engineering in Nano-Electronics

is forwarded for your information.

SUBMITTED FOR PUBLICATION TO (applicable only if report is manuscript):

DO NOT REMOVE LABEL BELOW
FOR IDENTIFICATION PURPOSES

Sincerely,

Dr. Geoffrey F. Strouse
University of California-Santa Barbara
Dept. of Chemistry
Santa Barbara, CA 93106

40413-LS-II

Title: Assembling Nano-Materials by Bio-Scaffolding: Crystal Engineering in Nano-Electronics
Contract: ARO - DAAD19-99-1-0193

Officer: Robert J. Campbell

2.b.4 Research Objectives and Goals: Developing high-density nano-electronics requires a cross-disciplinary approach for the formation of nano-scale arrays. We have successfully investigated the assembly of electronic materials based on 3-d super-lattices of semiconductor nano-particles connected by biological polymers (DNA, polypeptides). Bio-scaffolding of nano-scale materials is effectively the molecular analogue of amino-acid assembly into proteins, where tertiary structures are driven by the individual amino acid interactions. The proposed studies offer a glimpse into the design and development of a bio-electronic circuit coupling advanced materials and biological technologies to create a novel optical or electronic device. Engineering nano-structures using biological connectors (B) may allow technology that incorporates the capacity for self-wiring and self-healing.

2.b.5 Important Results: Next generation materials developed at the interface between traditional inorganic materials and biological polymers (DNA, polypeptides) can form the basis of novel nano-scale devices. Bio-assembly strategies may allow formation of high-density nano-electronic architectures possessing unique electronic properties. The tremendous advances in understanding, utilization and control of bio-materials, including DNA, provides a dramatically enhanced set of tools and techniques for the synthesis of novel materials with enhanced electronic functions. The most dramatic advances at this interface between inorganic and organic materials, between biological and electronic building blocks are yet to be made. Specifically, we have developed a non-lithographic methodology for constructing periodic structures composed of discrete 2-5 nm nanoscale materials (Au, CdSe) using double stranded DNA (14 – 20 mer oligomeric B-helix) and polypeptides (poly-alanine). We are currently completing the analysis of changes in the structure of the DNA or polypeptides accomplished by exposure to either site-specific DNA enzymes (e.g., methyl transferases such as MEcoRI) that produce specific structural changes in the DNA helix or ligand induced folding events in the polypeptides.

Two issues that are critical to bio-engineering of nanomaterial structures is bio-compatibility and bio-viability. Assembly of nanomaterials is largely dictated by steric and Van der Waals forces which are governed by the radii of the nanocrystal and the characteristics of the ligands passivating the materials and connecting them to, in this specific case, the biological molecule. A major caveat of bio-scaffolding of nano-materials is the incompatibility of inorganic materials in biological environments, and the domination of biological tertiary structure by the nanomaterial bulk. For biological materials, the contingencies of maintaining tertiary structure and catalytic activity in the presence of nanometer-sized particles is a potential fatal flaw in bio-scaffolded assemblies.

DNA Bio-Assembly. Our initial studies into these materials have been promising. We have demonstrated the assembly of both polymeric and dimeric structures using nanomaterials (Au,

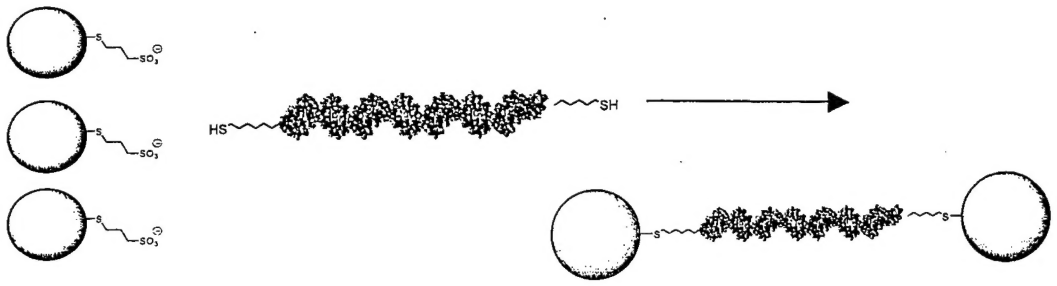


Figure 1: Assembly of CdSe Nanocrystals With DNA

CdSe) and a 40-mer DNA scaffold. In these materials the linkage is achieved by face selective capping of the nanomaterials by a hexyl thiol terminated 5' end of a 40-mer DNA single strand (Figure 1). The single strand modified nanoparticles show mobility in 5% Agrose gel-electrophoresis, allowing standard Bio-chemical methodologies to be utilized for the separation and purification of these materials. Preparation of the dimeric or polymeric structures is achieved by reaction of the single strands in annealing conditions.

DNA Bio-Compatibility. Bio-compatibility was analyzed in these samples by analysis of the separation distances observed in the TEM image. In analogy to the efforts of Mirkin, et al, large 3-dimensional constructs of 13 nm Au and DNA can be formed, however, the persistence length of the DNA is not maintained (Figure 2). This can be understood by inspecting the energetics of packing of the Au nanomaterials. The large van der Waals energy terms in 13 nm Au tends to dominate the assembly, giving rise to closely packed Au constructs, as observed in the TEM image. The E_{vdw} term should be minimized by reducing the Au radius. In Figure 3, the assembly of 1.4 nm Au-DNA dimeric structures provides a persistence length of ~12 nm, closer to the expected DNA structure for a 40-mer adopting an alpha-helical structure. The importance of the vdw contribution is further illustrated in constructs formed from DNA and CdSe. The CdSe-DNA-CdSe structure in the TEM image in Figure 4 arises from stoichiometric substitution of DNA onto the preformed 5.0 nm CdSe particles via a modification of the phosphate backbone with a thiol termination (P-O-$(CH_2)_6$-SH) at the 5' end of the 40-mer DNA strand. Due to the large footprint of the DNA double helix, we observe predominately single site substitution on the 5.0 nm CdSe nanocrystals. In the image the two CdSe nanomaterials image as individual

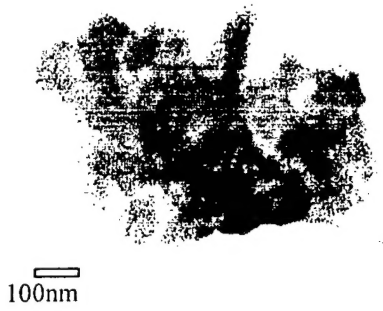
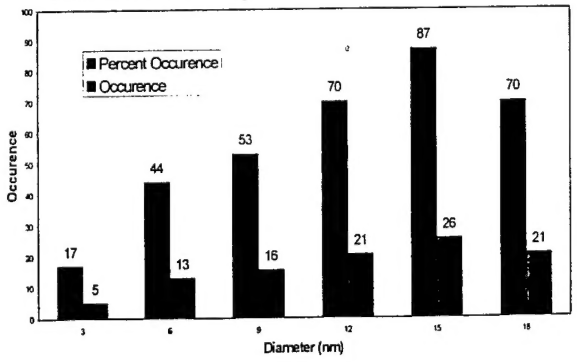


Figure 2: TEM image of 13.0 nm Au DNA construct.

Figure 3: Statistical Analysis of persistence length of DNA in 1.4 nm Au duplex DNA structures



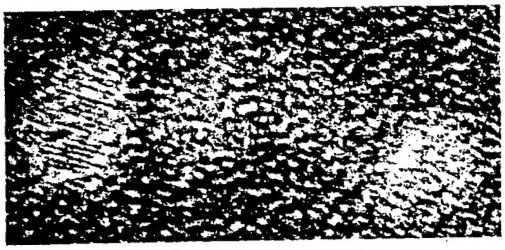
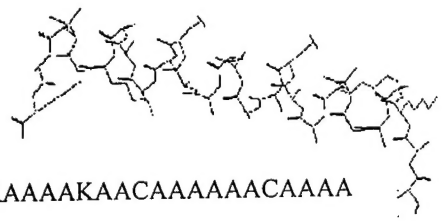


Figure 4: TEM image of 6.5 nm CdSe assembled by duplex DNA. The assembly illustrates Au plating DNA .

spheres coupled by a curved rod. The rod between the CdSe nanoparticles represents the direct wiring of the-nano-components with a gold rod. The gold rod wiring is achieved by of Au^{I} ions that are exposed to the DNA strand following the CdSe-DNA-CdSe assembly. The reduction of the Au^{I} ions results in formation of apparent Au wires over the DNA scaffolding. This shadowing technique provides a direct means to allow wiring of nano-scale components by non-lithographic methods. This may present a novel methodology for nanoscale wiring of individual nanocomponents utilizing bio-scaffolds in larger 3-d assemblies. The TEM images represent our initial studies on the formation of gold wires connecting individual CdSe nanoparticles assembled by bio-scaffolding.

Polypeptide Bio-Assembly. Bio-Assembly of $Au_{1.4}$ to proteins was accomplished through linkage to cysteines attached at site-specific mutations on polypeptides. Choice of cysteine modification arises due to its limited observation in inter-cellular proteins, as well as it the presence of a reactive mercapto functionality. The first polypeptide (Figure 5) was synthesized



AAKAAAAKAACAAAAAACAAAA

(A = ala; C = cys, K = lys)

Figure 5. Poly-alanine structure and Sequence

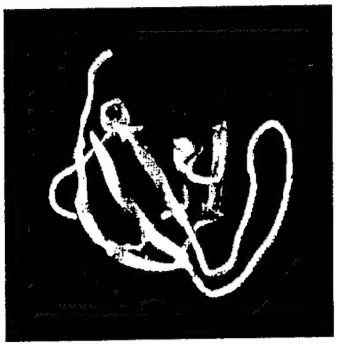


Figure 6. Ribbon structure of the catalytic domain of pIK3SH3, a phosphorylating protein

with two cysteines separated by a 6-sequence alpha helical region of poly-alanine which gives rise to 2.5 turns of an α helix, with a separation distance of ~ 1.5 nm. The polypeptide was chosen to maximize steric interactions in the protein in order to address bio-compatibility issues. In order to maintain protein solubility the terminal ends of the peptide are four lysine residues. The second polypeptide is a ~15kDa catalytic domain of the pIK3SH3, a sugar phosphorylating protein implicatedin the onset of Alzheimers desease (Figure 6). The pIK3SH3 catalytic domain was mutated at amino acid residues 19 and 68 by cysteines. Cysteine modified pIK3SH3 is chosen to address the effects of a 1.4 nm nanoparticle on tertiary folding and unfolding dynamics in naturally occurring proteins. The mutation sites serve as the attachment points for the nanocrystals, producing a separation distance of 8-9 nm in the folded state and a statistical distribution centered around 12-15 nm in the unfolded state. The domain was determined to possess its native tertiary form away from the main body of the protein by circular dichroism.

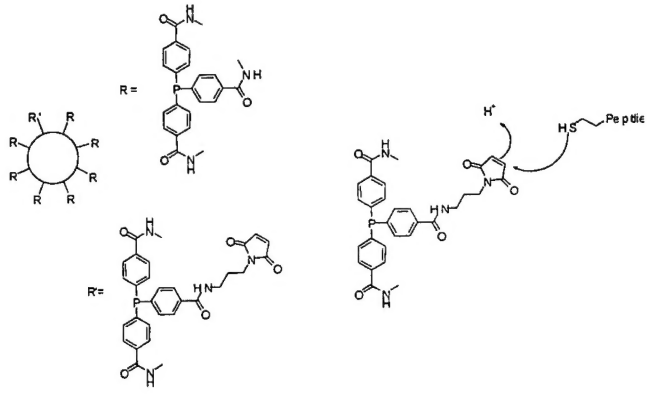


Figure 7. Monomaleimido Nanogold. A water-soluble phosphine layer, one of which carries the maleimido group, passivates the gold nanocrystal. The reaction mechanism the Michael Addition of the mercapto group of cysteine to the α, β unsaturated system.

Both polypeptides are treated with $Au_{1.4}$ possessing a single reactive based on a monomaleimido group. Based on absorption spectroscopy and TEM analysis two $Au_{1.4}$ nanoparticles are attached to the protein chains. The mechanism for attachment occurs through a Michael addition of the sulfhydryl group to the α, β unsaturated system of the maleimido group (Figure 7). The covalent bond that forms is stable at the pH ranges that are required to work with the peptides.

Polypeptide Bio-Compatibility. Bio-compatibility was analyzed in these samples by analysis of the separation distances observed in the TEM image. In the poly-alanine sample distances of 1.5

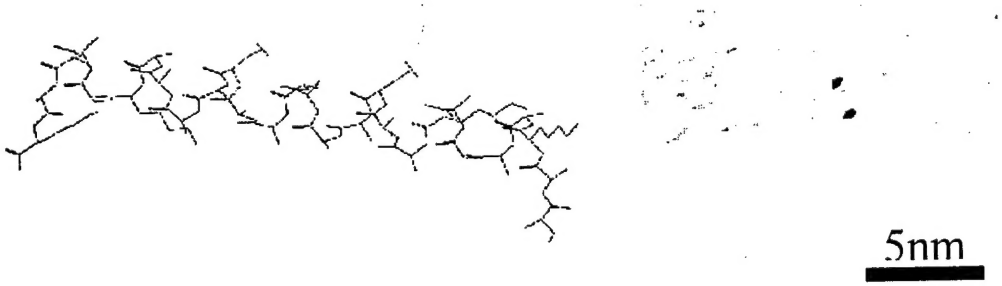


Figure 8. a) A cartoon representing gold-labeled polypeptide that contains regions of polyalanine and two cysteines. b) TEM image shows a gold-labeled polypeptide at infinite dilutions. The distance between the two gold crystals is approximately 1.5nm; the expected distance between the two cysteines.

nm separating the Au nanoparticles is consistent with appendage of two nanoparticles per protein (Figure 8). This range of separation is the expected distance of 6 amino acid residues that are in an alpha helical structure. Similarly, for the pIK3SH3 catalytic domain, the cysteines were labeled with $Au_{1.4}$. The structural morphology of the catalytic center appears to be maintained based on statistical analysis of the Au-Au separation (9nm) (Figure 9). The expected distance for a structurally intact catalytic domain is ~9nm. This suggests the steric and electrostatic

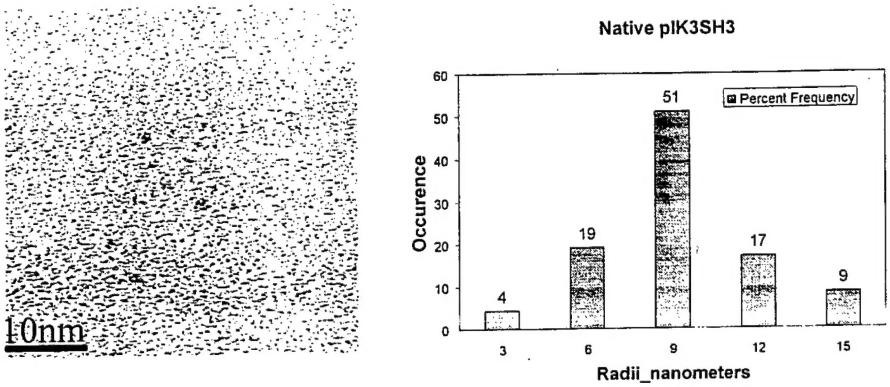


Figure 9. a) TEM image of a grid deposited with gold-labeled native peptide. b) The measured spatial distribution of gold nanocrystals labeled onto native peptide.

interactions of the Au nanoparticles with the polypeptide do not dominate the structural morphology of the polypeptide.

Polypeptide Bio-Viability. Bio-viability can be defined as the maintenance of bio-activity in the presence of the nano-materials. This is crucial for nano-architecture design using bio-scaffolds. In order to probe the issue of bio-viability, the effects of denaturation of the domain and the correlation of the spatial distance between gold particles was analyzed for the pIK3SH3 peptide. It is known that pIK3SH3 unfolds in the presence of high concentrations of urea due to the disruption of the hydrogen bonding regimes in the protein, and loss of the tertiary structure. Secondary structure however is maintained. This results in the observation of a larger statistical variation of the spatial relationship of the nanocrystals with a persistence length of ~9 nm arising

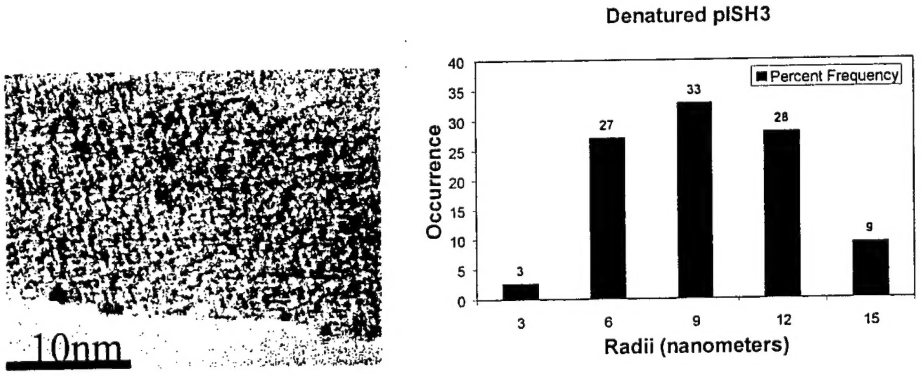


Figure 10. a) TEM image of a grid deposited with gold labeled denatured proteins. b) The measured spatial distribution of gold nanocrystals labeled onto denatured proteins.

from secondary structural effects. (Figure 10). As a control unreacted nano-particles were diluted to the appropriate concentration under the same conditions. Plotting of the distance between neighbors show a random arrangement of crystals with no apparent persistence length and at further dilutions show separation of crystals well beyond the length of the peptide. In this specific case the catalytic domain of the pIK3SH3 maintained its tertiary structure and showed that the both nano-material and polypeptide maintained their viability in similar environments.

Further studies of the interactions as a function of the nanomatertial type ands size are underway to provide further insight into the application of bio-scaffolding to non-lithographic assembly strategies in nano-electronics.

Conclusions:

Assembly of these structures represents our effort to apply engineering approaches using bio-scaffolding strategies for formation of nanoscale structures. We have shown that functionalized nanomaterials can be appended to polypeptides via cysteine or thiol labeling. These constructs are bio-compatible, thus allowing manipulation of the biological materials in aqueous environments without perturbation of the native tertiary structures. Furthermore, the protein-Au and DNA-Au or DNA-CdSE constructs maintain bio-viability. For instance, the labeling of the catalytic domain of the pI3SK3, a sugar phosphoralyting protein implicated in Alzheimers, the tertiary structure is unperturbed based on TEM analysis of the folded state. In the DNA constructs, nano-wiring of non-lithographically assembled CdSe materials has been demonstrated, providing a potential methodology for electronic architectures bridging the wealth of flexibility inherent in biological materials and the electronic potential of nano-scale materials. The assembly of nano-materials using biological scaffolding is attainable as long as the criteria of compatibility and viability are met.

2.b.6 Publications:

1) "Assembly of Nanomaterials Using Bio-Scaffolding." Yun, C.S.; Major, J.L.; Strouse, G.F. Mat. Res. Soc. Symp. Proc. in press 2001.

2) "Nanoscale wiring of bio-scafolded nano-structures." S. Yun, J. Major, G. Khitrov, N.O. Reich, G.F. Strouse, Chem. Mater. Manuscript in preparation.

2.b.7 Scientific Personnel

Graduate Students:

C. Steven Yun
G. Khitrov

Undergraduate Students:

Jody Major

2.b.8 Report of Inventions: None